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UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES

Ex parte MARK S. CHEE
and
JOHN R. STUELPNAGEL,
Appellants

Appeal 2009-000287
Application 09/513,362[1]
Technology Center 1600

Decided: September 17, 2009

Before CAROL A. SPIEGEL, ROMULO H. DELMENDO, and
RICHARD M. LEBOVITZ, *Administrative Patent Judges*.

SPIEGEL, *Administrative Patent Judge*.

DECISION ON APPEAL

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[1] Application 09/513,362 ("the 362 Application"), *Nucleic Acid Sequencing Using Microsphere Arrays*, was filed 25 February 2000 and claims benefit under 35 U.S.C. § 119 of the 22 October 1999 filing date of provisional applications 60/160,917 and 60/161,148, of the 18 October 1999 filing date of provisional application 60/160,027, of the 20 May 1999 filing date of provisional applications 60/135,123, 60/135,053, and 60/135,051, and the 20 April 1999 filing date of provisional application 60/130,089. The real party in interest is ILLUMINA, INC. (Appeal Brief filed 14 December 2007 ("App. Br.") at 1).

I. Statement of the Case

Appellants appeal under 35 U.S.C. § 134 from an Examiner's final rejection of all pending claims, claims 1-38 and 40-50 (App. Br. 3). An oral hearing was held May 20, 2009. We have jurisdiction under 35 U.S.C. §§ 6(b) and 134. We AFFIRM.

The subject matter on appeal is directed to microsphere or bead array based pyrosequencing methods and kits therefor. Claims 10 and 18 are illustrative and read (App. Br. Claims App'x. 28-30).

> 10. A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:
>
> a) providing [a] first hybridization complex comprising a first target sequence and a first sequencing primer that will hybridize to the first domain of said first target sequence,
>
> b) providing a second hybridization complex comprising a second target sequence and a second sequencing primer that will hybridize to the second domain of said second target sequence,
>
> wherein said first and second sequencing primers are covalently attached to microspheres distributed at discrete sites on a surface of a substrate, said discrete sites having an attached enzyme used to generate a signal from pyrophosphate;
>
> c) determining the identity of a plurality of bases at said target positions, wherein said determining comprises simultaneously extending said first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; and
>
> d) detecting the release of pyrophosphate (PPi) with said enzyme attached at said discrete sites within a common reaction chamber of said simultaneous extensions to determine the type of said first nucleotide

added onto said first and second sequencing primers, respectively.

18. A kit for nucleic acid sequencing comprising:
    a) a composition comprising:
        i) a substrate with a surface comprising discrete sites;
        ii) a population of microspheres distributed on said sites; wherein said microspheres comprise different capture probes, wherein said array is configured for simultaneous contact of said different capture probes with a common reaction chamber; and
        iii) an enzyme attached at said discrete sites, wherein said enzyme is used to generate a signal from pyrophosphate;
    b) a first extension enzyme; and
    c) dNTPs.

The Examiner has finally rejected the pending claims as unpatentable under 35 U.S.C. § 103(a) as follows:

(i) claims 1-16, 22-27, 31-38, 40-42, 44-47, 49, and 50 over Rothberg[2] and Walt[3] (FR[4] 5-12; Ans.[5] 4-11);

(ii) claim 17 over Rothberg, Walt, and Nyren[6] (FR 12; Ans. 11-12);

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[2] U.S. Patent 6,274,320 B1, *Method of Sequencing a Nucleic Acid*, issued 14 August 2001, based on application 09/398,833, filed 16 September 1999, to Rothberg et al. ("Rothberg").

[3] U.S. Patent 6,327,410 B1, *Target Analyte Sensors Utilizing Microspheres*, issued 4 December 2001, based on application 09/151,877, filed 11 September 1998, to Walt et al. ("Walt").

[4] Final Office Action mailed 30 May 2007 ("FR").

[5] Examiner's Answer mailed 17 March 2008 ("Ans.").

[6] International patent application WO 98/13523, *Method of Sequencing DNA*, published 2 April 1998 by Nyren et al. ("Nyren").

(iii) claims 18, 19, 28-30, 43, and 48 over Rothberg, Walt, Nyren, and Stratagene[7] (FR 12-16; Ans. 12-16); and,

(iv) claims 20 and 21 over Rothberg, Walt, Nyren, Stratagene, and Ross[8] (FR 16-17; Ans. 16-17). The Examiner also relies on Michael[9] and Ronaghi[10] as evidence of unpatentability (Ans. 2-3, 20, and 22).

According to the Examiner, Rothberg teaches attaching reactants of a pyrosequencing method to the surface of a fiber optic bundle, but does not teach using microspheres to attach the reactants (Ans. 4-9). According to the Examiner, Walt teaches microsphere-based analytical chemistry systems comprising optically identifiable populations of microspheres carrying bioactive agents which are randomly distributed on a fiber optic bundle or array and using the array for sequencing (Ans. 9-10). The Examiner concluded that it would have been obvious to combine the microspheres of Walt with the fiber optic bundle used in Rothberg's sequencing method to allow the generation of large fiber optic arrays comprising randomly distributed microspheres that can be encoded or decoded in order to provide a fast and inexpensive method compared to either the *in situ* synthesis or spotting techniques of the prior art (Ans. 10-11).

Appellants acknowledge that Walt describes the use of beads on a fiber optic surface but argue that, absent any articulated reason for using the beads in an array for the pyrosequencing method of Rothberg, the Examiner has failed to establish a

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[7] STRATAGENE 1988 CATALOG, "Gene Characterization Kits," 39 ("Stratagene").
[8] International patent application WO 91/06678, *DNA Sequencing*, published 16 May 1991 by Ross et al. ("Ross").
[9] Michael et al., "Randomly Ordered Addressable High-Density Optical Sensor Arrays," 70 *Analytical Chemistry* 1242-48 (1998) ("Michael").
[10] Ronaghi et al., "Real-Time DNA Sequencing Using Detection of Pyrophosphate Release," 242 *Analytical Chemistry* 84-89 (1996).

prima facie case of obviousness (App. Br. 14-15; Reply Br.[11] 1-3). Appellants further argue that Rothberg teaches away from bead usage, e.g., bead loss during washing limited the ability to sequence longer stretches of nucleic acid (App. Br. 15-17; Reply Br. 3-6). Appellants rely on the prosecution history of a continuation-in-part application of Rothberg, i.e., application 09/814,338 ("the Rothberg CIP application") to support their position that Rothberg not only believed that bead loss limited previous pyrosequencing methods, but also had to physically alter its substrate to allow for the use of beads (App. Br. 17-21; Reply Br. 6-7). In particular, Appellants rely on the following exhibits made of record in its March 8, 2007 response: the Rothberg CIP application,[12] Rothberg CIP Notice of Allowability,[13] Rothberg CIP Amendment and Response,[14] Margulies Declaration I,[15] and Margulies Declaration II.[16]

Thus, at issue is whether Appellants have shown that the Examiner failed to articulate a reason for combining the optically identifiable microspheres of Walt with the pyrophosphate sequencing method of Rothberg and/or that Rothberg teaches away from such a combination.

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[11] Reply Brief to Examiner's Answer filed 19 May 2008 ("Reply Br.").
[12] U.S. application 09/814,338, *Method of Sequencing a Nucleic Acid*, filed 21 March 2001, now U.S. Patent 7,244,559 B2, issued 17 July 2007, to Rothberg et al. ("the Rothberg CIP application"), which Appellants filed as Exhibit A.
[13] Notice of Allowability of application 09/814,338 mailed 7 February 2007 ("Rothberg CIP Notice of Allowability"), which Appellants filed as Exhibit B.
[14] Amendment and Response to the Non-Final Office Action Dated November 6, 2003 filed in the Rothberg CIP application on 23 April 2004 ("Rothberg CIP Amendment and Response"), which Appellants filed as Exhibit C.
[15] Declaration of Marcel Margulies, Ph.D., Under 37 C.F.R. § 1.132, dated 20 April 2004 ("Margulies Decl. I"), which Appellants filed as Exhibit D.
[16] Declaration of Marcel Margulies Under 37 C.F.R. § 1.132, dated 22 December 2006 ("Margulies Decl. II"), which Appellants filed as Exhibit E.

Appellants have presented a single set of patentability arguments. Therefore, we decide this appeal on the basis of claim 10. 37 C.F.R. § 41.37(c)(1)(vii).

II. Findings of Fact ("FF")

The following findings of fact are supported by a preponderance of the evidence of record.

A. The 362 application ("Spec.")

[1] According to the "Background of the Invention" section of the 362 application (Spec. 1:21-3:2), a pyrophosphate (PPi) is released during a DNA polymerase reaction as a nucleotriphosphate (dNTP) is added a growing nucleic acid chain (Spec. 2:14-16).

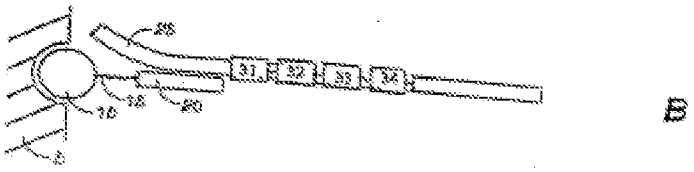
[2] Several known DNA sequencing systems, including Nyren, are based on detecting base (A, G, C, T) incorporation by measuring released PPi (Spec. 2:14-20).

[3] In one known method, for example,

> the four deoxynucleotides (dATP, dGTP, dCTP and dTTP; collectively dNTPs) are added stepwise to a partial duplex comprising a sequencing primer hybridized to a single stranded DNA template and incubated with DNA polymerase, ATP sulfurylase, luciferase, and optionally a nucleotide-degrading enzyme such as apyrase. A dNTP is only incorporated into the growing DNA strand if it is complementary to the base in the template strand. The synthesis of DNA is accompanied by the release of PPi equal in molarity to the incorporated dNTP. The PPi is converted to ATP and the light generated by the luciferase is directly proportional to the amount of ATP. In some cases the unincorporated dNTPs and the produced ATP are degraded between each cycle by the nucleotide degrading enzyme. [Spec. 2:21-29.]

[4] The invention of the 362 application is characterized as directed to methods using the detection of PPi generated during nucleic acid synthesis (pyrosequencing), particularly to methods using microsphere arrays (Spec. 1:17-19).

[5] The 362 application discloses several ways to attach a DNA template (target nucleic acid) to the surface of an array, such as an array formed on the surface of a biosensor (Spec. 1:18-19; 2, (as amended) ¶ 2). Figure 1B, for example, depicts a target nucleic acid 25 comprising a first portion (domain for which sequence information is not desired), which is hybridized to a nucleotide sequence primer 20 (which also serves as a capture probe), and an adjacent second portion containing nucleotide target positions 31, 32, 33, and 34 for which sequencing is desired. The sequence primer 20 is tethered to a microsphere 10 by means of a linker 15. The microsphere 20 is in a well (site) in the surface of a substrate 5 of an array. [Spec. amended 2, ¶ 2; 9:4-6.] Figure 1B is reproduced below.



{Figure 1B of the 362 application depicts one configuration for attaching a target sequence to a discrete site (well) on an array surface.}

[6] In one embodiment, the substrate, comprising microspheres with attached hybridization complexes of target nucleic acids and primers, is dipped or contacted with a reaction chamber containing a single type of dNTP, an extension enzyme, such as DNA polymerase, and the reagents and enzymes necessary to detect PPi (Spec. 10:19-21; 10:37-11:1; 12:13-16).

[7] If the dNTP is complementary to the base of the target position of the target nucleic acid, the dNTP is added (extending the sequence primer as a growing nucleic acid chain), thereby releasing PPi and generating detectable light. If the dNTP is not complementary to the base at the target position, no PPi is released and no light is generated. The process is repeated to obtain the sequence of the target position(s) on the target nucleic acid. (Spec. 12:16-23.)

[8] Preferably, the reaction chamber is washed between dNTP additions to remove unreacted dNTP and decrease background signal (Spec. 12:25-30).

[9] In a preferred embodiment, the enzymes used to detect PPi (the secondary or signal generating enzymes) are attached to sites on the array, e.g., the microspheres, preferably using flexible linkers (Spec. 14:34-15:2).

[10] Attaching the signal generating enzymes to the sites on the array is said to increase the concentration of the enzymes in the vicinity of the PPi detection reaction, thereby allowing use of less enzyme and faster reaction rates for detection (Spec. 15:2-4).

[11] In a preferred embodiment, the substrate is an optical fiber bundle or array (Spec. 24:18).

[12] According to the 362 application, at least one surface of the substrate is modified to contain discrete, individual sites for later association of microspheres such that the microspheres do not move during the course of the assay. The sites may be physically altered sites, e.g., wells or depressions, or chemically altered sites, e.g., spots of adhesive and sites with functional groups, such as amino, carboxy, oxo, and thiol groups, for covalently attaching microspheres. (Spec. 24:27-32; 25:28-26:8; 26:31-33.)

[13] When microsphere arrays are used, an encoding/decoding system must be used to correlate a functionality on the bead with its location since the beads are generally distributed randomly onto the substrate (Spec. 30:4-8).

[14] For example, a coding/decoding system may use a decoding binding ligand (DBL), generally directly labeled with an optical signature such as a fluorescent dye, that binds to either the capture probe or to identifier binding ligands (IBLs) attached to the beads (Spec. 30:22-30).

[15] The coding/decoding system allows nucleic acid synthesis to occur on the beads after random distribution on an array, thereby providing a faster and less expensive process as compared to either the *in situ* synthesis or spotting techniques of the prior art. After sequencing, the random array is decoded to determine the functionality of each bead (Spec. 30:8-22).

B. Rothberg

[16] Rothberg discloses a method of determining the sequences of target nucleic acid sequences (templates) attached to a solid substrate such as optical fiber bundle surfaces, wherein one or more of the surfaces have an array of anchored primers (capture probes) (Rothberg 2:62-3:6; 3:41-45; 5:6-16; 6:62-66).

[17] In some embodiments, the anchor primers are linked directly to the termini of the optical fibers which have been etched to form cavities therein which are, preferably, one-half to three times the diameter of the optic fiber (Rothberg 20:15-24).

[18] Rothberg also discloses a sequencing apparatus comprising, in one embodiment, (i) a perfusion chamber including a sealed compartment designed to allow laminar flow of solution, e.g., reagents and washes, over a substrate, such as a wafer formed from the termini of the optical fibers, (b)

the substrate wafer, and (c) a CCD imaging system (Rothberg 19:26-20:13; Figures 2 and 3).

[19] In use, an anchor primer is annealed to at least one nucleic acid template to form a hybridization complex and then a sequencing primer is annealed to the template and extended with a polymerase and a predetermined dNTP. If the predetermined dNTP is incorporated into the primer, PPi is generated and determined, e.g., by enzymatic conversion to detectable light using sulfurylase and luciferase. Additional predetermined dNTPs are added sequentially and the presence or absence of PPi generation associated with each synthesis reaction is determined. (Rothberg 3:18-45; 14:13-41, 50-52, 64-66; 16:7-8, 13-16; 17:1-12, 24-36.) Sulfurylase can be utilized to generate ATP with the liberated PPi; the thus produced ATP is converted to light using enzymatic reactions, such as luciferase. (Id. at 14:64-66; 16:7-20.)

[20] To increase the effective concentration of sulfurylase and to mitigate the generation of background light, the enzymes used to detect PPi maybe immobilized in the region of the nucleic acid template (Rothberg 27:58-62; 28:60-67).

[21] In addition to the above two tactics for optimizing the pyrosequencing analysis, Rothberg also suggests detecting only early light, increasing the concentration of sulfurylase in the reaction mixture, increasing the time it takes for the PPi to diffuse out of the reaction area by adding a viscosity increasing agent to the pyrosequencing reagents, and physically preventing the PPi from diffusing laterally, e.g., by increasing the depth of the etched cavities (Rothberg 27:46-52; 28:13-18, 24-41).

[22] Rothberg also provided the following discussion of the historical development of pyrophosphate sequencing:

> Solid-phase pyrophosphate sequencing was initially developed by combining a solid-phase technology and a sequencing-by-synthesis technique utilizing bioluminescence (see e.g., Ronaghi, et al., 1996, Real-time DNA sequencing using detection of pyrophosphate release. *Anal. Biochem.* 242: 84-89). In the solid-phase methodology, an immobilized, primed DNA strand is incubated with DNA polymerase, ATP sulfurylase, and luciferase. By stepwise nucleotide addition with intermediate washing, the event of sequential polymerization can be followed. A remarkable increase in signal-to-noise ratio was obtained by the use of α-thio dATP in the system. This dATP analog is demonstrated to be efficiently incorporated by DNA polymerase while being silent for luciferase, allowing the sequencing reaction to be performed in real-time. *In these early studies, sequencing of a PCR product using streptavidin-coated magnetic beads as a solid support was presented. However, it was found that the loss of beads during washing, which was performed between each nucleotide and enzyme addition, was the limiting factor to sequence longer stretches*. (Rothberg 21:14-34, emphasis added.)

C. Appellants' Evidentiary Exhibits from the prosecution of the Rothberg CIP Application

[23] The Rothberg CIP application provided the same discussion of the historical development of pyrophosphate sequencing quoted in fact 22 (Rothberg CIP application 39:12-24).

[24] According to the Rothberg CIP Amendment and Response and the testimony of Marcel Margulies, the Rothberg CIP application "expressly recognizes the

problem of bead/sample loss during the sequencing reaction" (Rothberg CIP Amendment and Response, p. 20, ll. 14-16; Margulies Decl. I ¶17).

[25] Marcel Margulies, Ph.D., testified on behalf of the exclusive licensee of the Rothberg CIP application that the superior performance of the Rothberg CIP application invention can be attributed to 1) compact wafers, 2) attachable optical fibers, 3) flow chamber and fluid means, and 4) specific fiber and well sizes (Margulies Decl. I ¶¶ 1, 2, 4).

[26] According to Dr. Margulies, continuous plunging of an optic fiber into nucleotide solutions would tend to dislodge any beads from the wells, whereas use of a flow chamber allows rapid and efficient delivery of sequencing reagents and washes (Margulies Decl. I ¶ 12-14).

[27] Dr. Margulies testified that using wells with a depth of one-half to three times the diameter of the optic fibers allows for more effective sequence analysis (Margulies Decl. I ¶¶ 17-18).

[28] According to Dr. Margulies,

> [w]ell depth is selected on the basis of a number of competing requirements in a nucleic acid sequencing application: (1) wells need to be deep enough for DNA-carrying beads to remain in the wells in the presence of convective transport past the wells; (2) the wells must be sufficiently deep to provide adequate isolation against diffusion of by-products from a well in which incorporation is taking place to a well where no incorporation is occurring; (3) they must be shallow enough to allow rapid diffusion of nucleotides into the wells and rapid washing out of remaining nucleotides at the end of each flow cycle to enable high sequencing throughput and reduced reagent use; and (4) they must not be so deep that it would be easy for more than one bead to fit in a well (Margulies Decl. II ¶8).

[29] The Examiner's reasons for allowing the Rothberg CIP application are reproduced *in toto* below:

> The diameter range, the depth, and well-depth of the claimed cavitated fiber optic wafer, said wafer being formed from a fused bundle of a plurality of individual optical fibers, are deemed non-obvious over the general teaching provided for by Chee et al. (of record), based on Margulies Declaration, as each of the above-mentioned parameters are critical to the laminar flow of the reaction reagents, allowing for rapid diffusion and washing out of the nucleotides, enabling a high cycle sequencing (see page 3 bottom paragraph to page 4, top paragraph; Margulies Declaration).
>
> Hence, the parameters are no longer deemed arbitrary, not involving routine optimization, as the parameters are specifically adapted to the way in which the disclosed invention operates. [Rothberg CIP Notice of Allowability p. 2, ¶¶ 1-2.]

D. Walt

[30] Walt describes an analytical system and method based on

> 1) the development of a bead-based analytic chemistry system in which beads, also termed microspheres, carrying different chemical functionalities may be mixed together while the ability is retained to identify the functionality of each bead using an optically interrogatable encoding scheme (an "optical signature"); and 2) the use of a substrate comprising a patterned surface containing individual sites that can bind or associate individual beads. This allows the synthesis of the bioactive agents (i.e. compounds such as nucleic acids and antibodies) to be separated from their placement on an array, i.e. the bioactive agents may be synthesized on the beads, and then the beads are randomly distributed on a patterned surface. Since the beads are first coded with an optical signature, this means

> that the array can later be "decoded", i.e. after the array is made, a correlation of the location of an individual site on the array with the bead or bioactive agent at that particular site can be made. This means that the beads may be randomly distributed on the array, a fast and inexpensive process as compared to either the in situ synthesis or spotting techniques of the prior art. Once the array is loaded with the beads, the array can be decoded, or can be used, with full or partial decoding occurring after testing . . . . (Walt 4:35-58.)

[31] Preferably, the substrate is a surface of a fiber optic bundle having one terminal end of the individual core fibers have been modified with small wells or cavities to provide discrete, individual sites for later association with the microspheres (Walt 5:57-63; 7:61-66).

[32] Preferably, the optical signature is a mixture of fluorescent dyes (Walt 13:25-26).

[33] The sites may be physically altered sites, e.g., wells or depressions that can retain the beads, or chemically altered sites, e.g., spots of adhesive and sites with functional groups, e.g., amino, carboxy, oxo, and thiol groups (Walt 5:61-6:2; 6:48-7:10).

[34] "[A] key component . . . is the use of a substrate/bead pairing that allows the association or attachment of the beads at discrete sites on the surface of the substrate, such that the beads do not move during the course of the assay" (Walt 7:50-54).

[35] For example, the beads are first distributed into the wells and subsequently exposed to an aqueous buffer which causes the beads to swell, thereby physically entrapping them in the wells (Walt 18:3-12). Alternatively, photoactivable attachment linkers, adhesives, or masks could be used to fix the beads inside the wells (*id.* 18:41-47).

[36] In one embodiment, the probes to detect a nucleic acid are in an array and used for sequencing by hybridization (Walt 21:25-28; 24:51-52).

E. Ronaghi

[37] Ronaghi is the reference cited by Rothberg in discussing early studies in pyrophosphate sequencing (*see* FF 22).

[38] According to Ronaghi, "[t]he decrease in signal due to loss and aggregation of beads during the washing procedure . . . has been compensated for" (Ronaghi 87, col. 2, ¶1).

[39] Ronaghi suggests that immobilizing the DNA template in a capillary could avoid the template loss observed for the paramagnetic beads (Ronaghi 88, col. 2, ¶2).

A discussion of Nyren, Ross, and Stratagene is not necessary to our decision.

III. Discussion

In order to establish a *prima facie* case of obviousness, the Examiner must show that each and every limitation of the claim is described or suggested by the prior art or would have been obvious based on the knowledge of those of ordinary skill in the art. *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988). Moreover, "[n]onobviousness cannot be established by attacking references individually where the rejection is based upon the teachings of a combination of references." *In re Merck & Co., Inc.*, 800 F.2d 1091, 1097 (Fed. Cir. 1986). It is also proper to "take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR Int'l Co. v. Teleflex, Inc.*, 550 U.S. 398, 418 (2007). *See also id.* at 421 ("A person of ordinary skill is also a person of ordinary creativity, not an automaton."). Furthermore, it is well settled that optimization of a result effective variable is within ordinary skill. *In re Peterson*, 315 F.3d 1325, 1330 (Fed. Cir. 2003).

Here, the Examiner found that Rothberg describes every step of the pyrosequencing method of claim 10 but using microspheres (Ans. 4-9). The Examiner found that Walt teaches randomly distributing populations of microspheres comprising a bioactive agent and an identifying optical signature on the surface of a fiber optic array which can be used for sequencing analysis (Ans. 9-10). The Examiner concluded that it would have been obvious to combine the microspheres of Walt with the fiber optic bundle used in Rothberg's sequencing method to allow the generation of large fiber optic arrays comprising randomly distributed microspheres that can be encoded or decoded in order to provide a fast and inexpensive method compared to either the *in situ* synthesis or spotting techniques of the prior art (Ans. 10-11). Thus, contrary to Appellants' argument (App. Br. 14-15; Reply Br. 1-3), the Examiner expressly articulated a reason for combining the teachings of Rothberg and Walt. Specifically, according to the Examiner, "[t]he advantage of using beads of Walt . . . is that the system provides an enormous flexibility and ease of use, as the fiber optic surface is simply filled with a different set of beads if an assay needs to be changed, saving time and money in building a sensor" (Ans. 19).

By way of emphasis, we note that Rothberg and Walt both relate to methods of determining nucleic acid sequences (FF 16, 19, 36). Rothberg and Walt both attach analytical reactants to the surface of fiber optic arrays (FF 16, 31) and both conduct analytical reactions in wells or cavities formed in the termini of the optical fibers (FF 17, 31). Walt teaches that since each population of its microspheres is individually encoded with its own optical signature, various populations of microspheres can be randomly distributed on the surface of the fiber optic array and decoded after loading, thereby providing a fast and inexpensive process as compared to either the *in situ* synthesis or spotting techniques of the prior art (FF

30). It is these benefits offered by the microspheres of Walt that the Examiner articulates as a reason for combining the teachings of Rothberg and Walt (Ans. 18-19).

Furthermore, while there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness, "the analysis need not seek out precise teachings directed to the specific subject matter of the challenged claim, for a court can take account of the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR*, 550 U.S. at 418.

> When a work is available in one field of endeavor, design incentives and other market forces can prompt variations of it, either in the same field or a different one. If a person of ordinary skill can implement a predictable variation, § 103 likely bars its patentability. For the same reason, if a technique has been used to improve one device, a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill. *Id.* at 417.

Thus, although Appellants complain that the Examiner did not articulate a reason to combine pyrophosphate sequencing with beads (App. Br. 15; Reply Br. 2), a precise teaching is unnecessary because it was well-established that pyrophosphate sequencing was conventional (FF 2, 3, 19) and microspheres (beads) had been suggested by Walt (FF 36) as suitable for carrying out sequencing. Therefore, in this case, combining the microsphere-based analytical system of Walt with the pyrosequencing method of Rothberg is no more than the predictable use of prior art elements according to their established functions.

Appellants also argue that Rothberg teaches away from bead usage because in early studies loss of beads during washing limited the ability to sequence longer stretches of nucleic acid (App. Br. 15-17; Reply Br. 3-6) (FF 22). However,

> [a] reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant. The degree of teaching away will of course depend on the particular facts; in general, a reference will teach away if it suggests that the line of development flowing from the reference's disclosure is unlikely to be productive of the result sought by the applicant.

In re Gurley, 27 F.3d 551, 553 (Fed. Cir. 1994). Here, the Examiner contends that (i) Ronaghi is discussing homogeneous assays where the microspheres are suspended in solution, (ii) the claims are not limited to sequencing nucleic acids of any particular length, (iii) Ronaghi suggests immobilizing the DNA template to prevent it from being washed away, and (iv) Walt teaches a number of ways to immobilize microspheres in the wells (Ans. 20-22).

While we do not find factual support for the Examiner's first contention in the record before us, we do agree with the Examiner's remaining contentions. By way of emphasis we note that the rejection is based on the combined teachings of Rothberg and Walt, not just Rothberg. We also note that Walt not only expressly uses substrate/bead pairings such that the beads do not move during the course of the assay (FF 34), but also provides a number of ways by which the beads can be covalently or non-covalently immobilized within the wells of the substrate (FF 35). Appellants' rebuttal evidence drawn from the prosecution history of the Rothberg CIP application does not convince us otherwise. At issue is what the combined teachings of Rothberg and Walt would have suggested to one of ordinary skill in the art, not to the inventors of the Rothberg CIP application. Allowance of the Rothberg CIP application was based on a different record than the one before us. For example, Dr. Margulies offered no opinion regarding the disclosure of Walt. In addition, both Rothberg and the Rothberg CIP application provided ample,

similar teachings on how to optimize assay parameters (*compare* FF 20-21 with FF 27-28). Finally, Appellants cite no authority holding that the Board is bound by the patentability determinations of an Examiner, particularly on a different record in a different case.

In conclusion, Appellants have not shown that the Examiner failed to articulate a reason for combining the optically identifiable microspheres of Walt with the pyrophosphate sequencing method of Rothberg or that Rothberg teaches away from such a combination. Consequently, we sustain the rejection of claims 1-38 and 40-50 under § 103.

IV. Order

Upon consideration of the record, and for the reasons given, it is

ORDERED that the decision of the Examiner rejecting claims 1-16, 22-27, 31-38, 40-42, 44-47, 49, and 50 over Rothberg and Walt is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner rejecting claim 17 over Rothberg, Walt, and Nyren is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner rejecting claims 18, 19, 28-30, 43, and 48 over Rothberg, Walt, Nyren, and Stratagene is AFFIRMED;

FURTHER ORDERED that the decision of the Examiner rejecting claims 20 and 21 over Rothberg, Walt, Nyren, Stratagene, and Ross is AFFIRMED; and,

FURTHER ORDERED that no time period for taking any subsequent action in connection with this appeal may be extended under 37 C.F.R. § 1.136(a).

AFFIRMED

saw

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